201- 14298

Nguyet Phan

02/13/2003 06:44 AM

To: NCIC HPV
cc:
Subject: Crude BHMT HPV robust summary/test plan document

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Nguyet Phan
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----- Forwarded by Nguyet Phan/DC/USEPA/US on 02/13/03 06:36 AM -----

Edwin L Mongan <Edwin.L.Mongan-1@USA.dupont.com> on 02/12/2003 09:24:54 AM

To: oppt.ncic@epamail.epa.gov, Rtk Chem/DC/USEPA/US@EPA
cc: Georgia R Pugh <Georgia.R.Pugh@usa.dupont.com>, Jim_Keith@americanchemistry.com, Rauckman@toxicsolutions.com, Kathleen E O'Keefe <Kathleen.E.O-Keefe@usa.dupont.com>

Subject: Crude BHMT HPV robust summary/test plan document

Dear Sir or Madam,

Attached to this message is a .pdf file containing a cover letter, a Robust Data Summary and a Test Plan for the chemical Hexanedinitrile hydrogenated, high-boiling fraction, CAS# 68411-90-5, also known as crude BHMT, for the HPV Challenge Program. This chemical is jointly sponsored by E.I. du Pont de Nemours and Company, Inc. and Solutia, Inc. in the HPV Challenge Program. Please post this information on the EPA HPV Challenge website. I have also attached a word version of the cover letter in addition to the signed copy of the cover letter in the .pdf file.

Regards,

Edwin L. Mongan
Manager, Environmental Stewardship
DuPont Company

(See attached file: Crude BHMT with coverletter.pdf)(See attached file: BHMT Amines.doc) http://www.DuPont.com/corp/email_disclaimer.html

Crude BHMT with coverletter.pdf   BHMT Amines.doc

Safety, Health & Environment Excellence Center
1007 Market Street, DuPont 6082
Wilmington, DE 19898
302-773-0910 (Office) – 302-774-3140 (Fax)

February 11, 2003

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 2216

Attn: Chemical Right-to-Know Program

Dear Administrator Whitman,

E. I. du Pont de Nemours & Company, Inc. and Solutia, Inc. are pleased to submit the proposed test plan along with the robust summary for Hexanedinitrile hydrogenated, high-boiling fraction, CAS# 68411-90-5. This chemical is also known as Crude BHMT, BHMT Amine and Amines Bottoms. DuPont and Solutia understand there will be a 120-day review period for the test plan and that all comments received by the EPA will be forwarded to us for consideration.

This submission includes one electronic copy in .pdf format.

Please feel free to contact either DuPont or Solutia with any questions or concerns you may have with regards to this submission at Edwin.L.Mongan-1@usa.dupont.com or Elmer Rockman, at Rauckman@toxicsolutions.com.

Sincerely,

E. I. du Pont de Nemours & Company, Inc.

Manager, Environmental Stewardship
DuPont Safety, Health & Environment

Solutia, Inc.

Elmer Rauckman, PhD, DABT
Consulting Toxicologist

Cc: Charles Auer – U.S. EPA
Office of Pollution Prevention & Toxics
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

201-14298A

(February 11, 2003)

ROBUST SUMMARY FOR CRUDE BHMT

Summary

Crude BHMT is the high-boiling fraction from the distillation of hexamethylene diamine. Because it is the residue of a distillation and the processing times and conditions are adjusted to maximize the yield of hexamethylene diamine, it is an inconsistent mixture. Crude BHMT is also known as:

BHMT Amine;
Amines Bottoms;
CAS NO 68411-90-5;
Hexanedinitrile, hydrogenated, high-boiling fraction; and
Adiponitrile, reaction product with hydrogen, high-boiling fraction;

It has a variable composition (dry weight basis) of ~50-70% bis-hexylmethylenetriamine (BHMT), ~20-35% oligomeric amines, ~0-10% C_{10} amines, ~0-10% hexamethylenediamine, ~0-10% caprolactam, ~0-5% adiponitrile, and ~0-5% 6-aminocapronitrile, and small amounts of related compounds.

Available data are presented in this document on crude BHMT and purified BHMT (the main component of the mixture).

Chemical Name	CAS Registry Number	Structure
Crude BHMT	68411-90-5	Variable mixture
BHMT	143-23-7	$H_2N-(CH_2)_6-NH-(CH_2)_6-NH_2$

The scientific literature was searched and summarized (Table 1). Studies were obtained and evaluated for adequacy. Robust summaries were developed for adequate studies addressing specific SIDS endpoints. Summaries were also developed for studies either considered not adequate but provided information of relevance for hazard identification and evaluation, or covered non-SIDS endpoints (Appendices A and B).

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Table 1: Matrix of Available and Adequate Data on Crude BHMT and BHMT

	Crude BHMT	BHMT
PHYSICAL/CHEMICAL CHARACTERISTICS		
Melting Point	√	√
Boiling Point	√	√
Vapor Pressure	√	√
Partition Coefficient (Log Kow)	–/*	√
Water Solubility	√	√
ENVIRONMENTAL FATE		
Photodegradation	√	√
Stability in Water	√	√
Transport (Fugacity)	–/*	√
Biodegradation	–/*	√
ECOTOXICITY		
Acute Toxicity to Fish	–/*	√
Acute Toxicity to Invertebrates	–/*	√/**
Acute Toxicity to Aquatic Plants	–/*	√/**
MAMMALIAN TOXICITY		
Acute Toxicity	√	√
Repeated Dose Toxicity	√	###
Developmental Toxicity	√	###
Reproductive Toxicity	√/*	###
Genetic Toxicity Gene Mutations	√	√
Genetic Toxicity Chromosomal Aberrations	√	###

√ = Data are available and considered adequate.
√/* = Data available on reproductive organs in a repeated dose study.
√/** = Modeled data are available, but no empirical data are available.
– = No data available, or available data considered inadequate.
/* = No studies were available; however, we expect similar results to BHMT.
= No studies were available; however, we expect similar results to crude BHMT.

Evaluation of Data

The available adequate data were broken out by discipline (physical chemical, environmental fate, ecotoxicology, and mammalian toxicology).

Crude BHMT and BHMT have similar physical chemical properties due to BHMT being a major component for crude BHMT. Available data (Table 2) correlate well and validate the proposal to use the BHMT data to aid in evaluation of crude BHMT toxicity.

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Table 2: Physical and Chemical Characteristics

	Crude BHMT	BHMT
Physical Appearance	Brown viscous semi-liquid or paste	White to slight yellow solid, shipped in bulk as a warm liquid
Water Solubility	1%	10.8 g/L (Estimated)
Melting Point	32-34°C	33-36°C
Boiling Point	Circa 249°C @ 100 mm Hg	220°C @ 20 mm Hg
Vapor Pressure	7 mm Hg @ 180°C	82.5 mm Hg @ 250°C
Density/ Specific Gravity	0.93-0.97 (Specific gravity)	No Data
Octanol-Water Partition Coefficient (Log Kow)	No Data	1.8 (Estimated) (base form)

Crude BHMT is a brown viscous liquid/semi-solid with an ammonia-like odor. Crude BHMT melts at 32-34°C, has a listed boiling point of 249°C, specific gravity of 0.93-0.97, water solubility of 1%, and a listed vapor pressure of 7 mm Hg at 180°C. BHMT has similar properties, in that it melts at 33-36°C, has a boiling point of 220°C, and vapor pressure of 82.5 mm Hg at 250°C.

Data on environmental fate are not available for crude BHMT; however, limited data on BHMT are available. As a complex and variable mixture, crude BHMT cannot be modeled for environmental fate. Inspection of their chemical structures and a review of estimated physical-chemical properties and environmental-fate characteristics, based on output from EPIWIN 3.05 modeling software (Syracuse Research Corporation), indicate that the known components of crude BHMT do not exhibit a potential to be persistent and/or bioaccumulative in the environment. BHMT is rapidly biodegraded when tested following standard protocols. At environmental pH, it is expected to be fully ionized and show little tendency for bioaccumulation. Based on Level III fugacity modeling using a standard emission scenario, BHMT is expected to partition primarily into soil and water. Inspection of the chemical structure and application of chemical principles indicate that BHMT will not readily hydrolyze in water or be subject to aqueous photolysis.

Table 3: Environmental Fate

	Crude BHMT	BHMT
Bioaccumulation*	No Data	BCF = 4.8 (base form only)
Biodegradation	No Data	Ready biodegradable
Fugacity*	No Data	(base form) Air 0% Water 25.2% Soil 74.7% Sediments 0.1%
* = Modeled data		

No data are available on the aquatic toxicity of crude BHMT. Data available indicate that BHMT is of moderate concern for toxicity to fish. ECOSAR modeling data are in agreement with the empirical data for fish. No empirical data for either crude BHMT or BHMT are available for invertebrates and algae. Therefore, short-term aquatic toxicity tests are proposed for *Daphnia magna* and *Selenastrum*.

Table 4: Aquatic Toxicity

	Crude BHMT	BHMT
Log Kow	No Data	1.8 (E)
Toxicity to Fish (48-hour LC_{50} value)	No Data	76 mg/L (N) 79.6 mg/L (E)
Toxicity to Invertebrates (48-hour EC_{50} value)	No Data	5.7 mg/L (E)
Toxicity to Algae (96-hour EC_{50} value)	No Data	10.1 mg/L (E)
E = estimated value, N = value based on nominal test concentrations		

Acute toxicity data indicates that crude and pure BHMT exhibit similar acute toxicity (Table 5). Crude BHMT was slightly toxic via the acute oral route with a reported acute lethal dose (ALD) in rats of 1500 mg/kg in one acute study and an LD_{50} in rats of 450 mg/kg in another study. BHMT also has an ALD in rats of 1500 mg/kg. Crude BHMT was considered to be at worst moderately toxic via the acute route (ALD > 200 mg/kg). Crude BHMT is corrosive to skin and a severe eye irritant.

Table 5: Acute Mammalian Toxicity

	Crude BHMT	BHMT
Oral	ALD = 1500 mg/kg LD_{50} = 450 mg/kg	ALD = 1500 mg/kg
Inhalation LC_{50}	6-hour > vapor saturation at ambient temperature	No Data
Dermal ALD	> 200 mg/kg > 126 and < 200 mg/kg	No Data
Dermal Irritation	Corrosive	No Data
Eye Irritation	Severe irritant	No Data
Dermal Sensitization	No Data	No Data

A summary of available data on repeated dose, developmental, and reproductive toxicity is shown in Table 6. Crude BHMT has been tested in a 13-week oral gavage study in rats. In this study, mortality was observed in 1/15 males and 1/15 females in the high dose group (120 mg/kg/day) and 1/15 males at the mid-dose group (50 mg/kg/day, death not considered treatment-related). Other toxic effects observed during the study included respiratory rales, decreased body weights, reduced food consumption, and an increase in segmented neutrophils. The NOEL for this study was 50 mg/kg/day for both sexes. Crude BHMT was also studied in a 13-week inhalation study in rats. A NOEL was not determined for this study as the lowest concentration level (10 mg/m^3) exhibited lesions in the respiratory tract, with target organs of the nasal passages, trachea, and lungs. BHMT was also tested in a developmental toxicity study in rats at dose levels of 50, 100, and 250 mg/kg. BHMT did not produce fetal effects at dose levels lower than where maternal effects existed; therefore, it was not considered to be a specific developmental toxin. Although no reproductive toxicity tests were conducted on either compound, no effect on testes or ovary weights were observed in 13-week oral and 13-week inhalation toxicity studies in rats. No microscopic findings were observed in the reproductive organs (ovaries, testis, epididymis, uterus, and/or vagina) of the high-dose group in either study.

Table 6: Repeated Dose, Developmental, and Reproductive Toxicity

	Crude BHMT	BHMT
Repeated Dose Toxicity (NOAEL)	NOEL = 50 mg/kg in a 13-week oral study NOEL <10 mg/m^3 in a 13-week inhalation study	No Data
Developmental Toxicity	Not a developmental toxin	No Data
Reproductive Toxicity	No effect on reproductive organs in subchronic studies.	No Data

Crude BHMT and BHMT were not mutagenic when tested in *Salmonella* and *E. coli.* Additionally, crude BHMT was negative in an *in vitro* hepatocyte DNA repair assay, negative in an *in vitro* CHO/HGPRT cell gene mutation assay, and negative in an *in vivo* bone marrow chromosome aberration study.

Table 7: Genetic Toxicity

	Crude BHMT	BHMT
Mutagenic Activity in Bacterial Systems	Negative	Negative
Chromosome Aberrations	Negative	No Data

Overall, the toxicologic database for crude BHMT is fairly robust. In areas where data gaps exist for crude BHMT, data for BHMT are available. Because BHMT is the primary component of crude BHMT, it is reasonable to conclude that the crude BHMT would behave similarly to BHMT in the areas where data gaps are evident for the crude material: log Kow, stability in water, fugacity, biodegradation, and acute toxicity to fish. While we expect crude BHMT to have similar toxicity values to the modeled data presented for BHMT for acute toxicity to invertebrates and algae, there is no empirical data available to support the modeled BHMT data. Therefore, acute aquatic toxicity tests are proposed for *Daphnia magna* and *Selenastrum* (see Table 8). A 48-hour static *Daphnia magna* toxicity test with crude BHMT following OECD Guideline 202 is recommended, as well as an acute toxicity test to algae with crude BHMT following OECD Guideline 201.

(February 11, 2003)

Table 8: Proposed Test Plan

	Crude BHMT
Acute Toxicity to Invertebrates	+
Acute Toxicity to Aquatic Plants	+
+ = Testing recommended.	

Exposure Assessment for Crude BHMT – DuPont and Solutia

Crude BHMT is a co-product produced in the manufacture of hexamethylenediamine (HMD). Crude BHMT is sold to several customers for use as an asphalt anti-stripping agent, a chelating agent for water treatment, or a curing agent for epoxy resins. DuPont and Solutia practice Responsible Care and assess the ability of potential customers to safely handle crude BHMT prior to commencing a commercial relationship. The Product Stewardship System works with customers to understand their applications and any issues associated with PPE (personal protective equipment), safety equipment (safety showers, eyewash stations, ventilation needs, etc.), storage concerns, disposal requirements, and MSDS questions.

Crude BHMT is manufactured at three DuPont and one Solutia facilities. The potential for exposure is the greatest during truck loading for production and during equipment breaks for maintenance. The sites can have from 430 to 2000 personnel working (construction, contractor, and plant employees). The areas where the substance is manufactured will have 20 to 40 operators during normal operations and 60 people during a shutdown or major construction activity. Two of the 3 DuPont sites ship their substance to the third site or a toller to refine the crude BHMT. Solutia only sells the crude product directly from the one facility.

The sites and toller have effective safety, health, and environmental practices and procedures in addition to engineering controls, environmental controls, and personal protective equipment to control exposure. Adequate safety equipment, such as safety showers, eyewash fountains, and washing facilities, are available in the event of an occupational exposure. DuPont and Solutia assess the ability of a toller to manage the risk of exposure prior to signing a contract. The DuPont contract requires that any incidents must be reported to DuPont.

Individuals handling crude BHMT should wear safety glasses and impervious clothing to prevent any contact with this product, such as gloves, apron, boots, or whole bodysuit made from neoprene, as appropriate. When the possibility exists for eye and face contact due to splashing or spraying of material, individuals handling crude BHMT should wear coverall, chemical splash goggles, and face shield. This material does not have established exposure limits. The following DuPont recommendations are based on the fact that similar amines typically have exposure limits of 1 to 10 ppm. Half-face air-purifying respirators should be used where airborne concentrations range up to 10 ppm. Full-face air-purifying respirators should be used where airborne concentrations range between 10 and 50 ppm. A positive-pressure air-supplied respirator should be used where airborne concentrations are expected to exceed 50 ppm. Final determination of appropriate respiratory protection equipment should be made by a qualified safety or industrial hygiene professional. If there is potential contact with hot/molten material, wear heat resistant clothing and footwear.

No occupational exposure limits have been established. Air monitoring has not been conducted on crude BHMT.

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APPENDIX A

(February 11, 2003)

Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

1.0 Substance Information

CAS Number:	68411-90-5
Chemical Name:	Reaction Product (adiponitrile/hydrogen) high-boiling fraction
Structural Formula:	Crude BHMT is a variable composition of ~50-70% BHMT, ~ 20-35% oligomeric amines, ~0-10% C_{10} amines, ~0-10% hexamethylenediamine, ~0-10% caprolactam, ~0-5% adiponitrile, ~0-5% 6-aminocapronitrile, and small amounts of related compounds.
Other Names:	Adiponitrile, reaction product with hydrogen, high boiling fraction Amine Bottoms Crude BHMT BHMT Amine BHMT Amine 60% Hexanedinitrile, hydrogenated, high boiling fraction Reaction product (adiponitrile-hydrogen)
Exposure Limits:	None available.

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	32-34°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1996). Material Safety Data Sheet No. 6083CR (July 11).
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point: None Found.

2.2 Boiling Point

Value:	Listed as 249°C @ 100 mm Hg on MSDS sheets, but actually varies over a wide range depending on the actual composition.
Decomposition:	Yes
Pressure:	100 mm Hg
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (2002). Material Safety Data Sheet No. 6039CR (March 29). Solutia Inc. (2002). Material Safety Data Sheet No. 00237 (August 17).
Reliability:	Not assignable because limited study information was available.

Additional References for Boiling Point:

EPIWIN 3.05 lists 220°C at 20 mm Hg as an experimental value match for the material.

MPBPWIN (v 1.40) estimates the BP at 760 mm as 323°C using the adapted Stein and Brown method.

2.3 Density

Value:	0.93-0.97 (Specific gravity), variable with composition
Temperature:	Not Applicable
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	DuPont Co. (2002). Material Safety Data Sheet No. 6039CR (March 29).
Reliability:	Not assignable because limited study information was available.

Additional References for Density: None Found.

2.4 Vapor Pressure

Value:	Variable, but listed as 7 mm Hg @ 180°C
Temperature:	180°C
Decomposition:	No Data

Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (2002). Material Safety Data Sheet No. 6039CR (March 29).
Reliability:	Not assignable because limited study information was available.

Additional References for Vapor Pressure:

MPBPWIN (v 1.40) estimates the VP at 25°C as 0.00047 mm Hg using the modified Grain method. At 180°C this program estimates the VP to be 4.26 mm Hg using the modified Grain method.

2.5 Partition Coefficient (log Kow): No Data.

2.6 Water Solubility

Value:	1% (by weight). Water solubility is variable, depending on the material's composition and the pH and hardness of the water.
Temperature:	25°C
pH/pKa:	No Data
Method:	Increments of crude BHMT were added to a known weight of water until the cloud point (haze or precipitation occurs) was reached. The weight that was in the solution (based on being just under that point) was calculated.
GLP:	Unknown
Reference:	DuPont Co. (2002). Unpublished data, "Water Solubility Test" (October 31).
Reliability:	Not assignable because limited study information was available.

Additional References for Water Solubility:

WSKOW (v1.40) estimates the water solubility of the base form to be 1084 mg/L based on the Ko/w and molecular weight.

3.0 Environmental Fate

3.1 Photodegradation:

Concentration:	No Data
Temperature:	No Data
Direct Photolysis:	The BHMT component of the crude BHMT is not expected to be subject to aqueous photodegradation.
Indirect Photolysis:	Atmospheric degradation:

AOP Program (v1.90) Results:

SMILES : NCCCCCCNCCCCCCN
CHEM : BHMT
MOL FOR: C12 H29 N3
MOL WT : 215.39

SUMMARY (AOP v1.90): HYDROXYL RADICALS
Hydrogen Abstraction = 54.0405×10^{-12} cm^3/molecule-sec
Reaction with N, S and -OH = 105.0000×10^{-12} cm^3/molecule-sec
Addition to Triple Bonds = 0.0000×10^{-12} cm^3/molecule-sec
Addition to Olefinic Bonds = 0.0000×10^{-12} cm^3/molecule-sec
Addition to Aromatic Rings = 0.0000×10^{-12} cm^3/molecule-sec
Addition to Fused Rings = 0.0000×10^{-12} cm^3/molecule-sec

OVERALL OH Rate Constant = 159.0405×10^{-12} cm^3/molecule-sec
HALF-LIFE = 0.067 Days (12-hr day; 1.5×10^6 OH/cm^3)
HALF-LIFE = 0.807 Hours

Breakdown Products: No Data
Method: Inspection of chemical structure and AOP Calculation
GLP: Not Applicable
Reference: Harris, J. C. (1990). Rate of Aqueous Photolysis. Chapter 8 In Lyman, W. J. et al. (eds.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC.

AOP v1.90 computer program as found in EPIWIN 3.05, Syracuse Research Corporation, Syracuse NY
Reliability: Estimate based on known qualitative structure-activity relationships.

Additional References for Photodegradation: None Found.

3.2 Stability in Water:

Concentration: Not Applicable
Half-life: Not Applicable
% Hydrolyzed: The BHMT component of the crude BHMT is not expected to readily hydrolyze in water. Amines are considered "non-hydrolysable".
Method: Inspection of chemical structure.
GLP: Not Applicable
Reference: Harris, J. C. (1990). Rate of Aqueous Hydrolysis. Chapter 7 In Lyman, W. J. et al. (eds.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC.

Reliability: Estimated value based on established chemical principle.

Additional References for Stability in Water: None Found.

3.3 **Transport (Fugacity):** No data due to its complex and variable composition.

3.4 **Biodegradation:** No Data.

3.5 **Bioconcentration:**

Value: No data were available on the crude BHMT mixture
Individual component estimate:
BHMT(neutral): BCF = 4.8
BHMT(ionized, single nitrogen): BCF = 3.2

Method: Modeled. BCFWIN v. 2.4 module of EPINWIN v3.05 (Syracuse Research Corporation). BCFWIN estimates the bioconcentration factor (BCF) of an organic compound using the compound's log octanol-water partition coefficient (Kow) with correction factors based on molecular fragments.
GLP: Not Applicable
Reference: "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient", SRC TR-97-006 (2nd Update), July 22, 1997; prepared for: Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-0012; prepared by: William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup and Sybil Gouchie; Syracuse Research Corp.
Reliability: Estimated values based on accepted model.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish:

Type: **48-hour LC_{50}**
Species: Orfe, *Leuciscus idus melanotus*
Value: 76 mg/L
Method: The procedure used in the test was based on the following guideline:

German Standard "Deutsches Einheitsverfahren" DIN 38 412, Part 15.

The effects of the test substance on *Leuciscus idus melanotus* within 48 hours were examined and compared to a negative control. Ten fish were exposed to various concentrations of the test substance (0, 18, 32, 58, and 100 mg/L) in water without using any solubilising agent. The test was performed using a static test procedure. Two hours before test start, 15 L of each concentration was prepared and distributed in the test containers. These solutions were stored under test conditions until the test started. After this precoating time, the solutions were replaced by freshly prepared solutions and 10 animals were introduced into the test containers (1 test container for each concentration).

The main test criteria were the mortalities after 24 and 48 hours in each solution. Those animals showing no reaction within a few seconds after touching the caudal peduncle were considered to be dead. Dead animals were removed at the observation times and test containers were exchanged and cleaned. Other significant effects as compared to controls were also documented.

Ambient air was pumped by means of an aquarium aerator through silicon rubber tubes and "flow-out stones" into the aquaria. The airflow was adjusted in order to maintain an oxygen concentration of >80% of saturation. No feeding occurred during the test. Sixteen hours of light and 8 hours of darkness were provided during the test. Water hardness was 2.2 mole $CaCO_3$/L.

The dilution water was aerated until a constant pH value (8.0±0.5) was reached.

GLP: Yes

Test Substance: BHMT, purity >92% by wt.

Results: After evaluation of the results with a "Probit" method, the following toxicity data were obtained:

NOEC = 58 mg/L (nominal);
0% mortality at 58 mg/L (nominal);
LC_{50} = 76 mg/L (nominal);
100% mortality = 100 mg/L (nominal).

No other effects of the test substance compared to the control were observed.

The pH was 8.4 (concentration not specified) when measured at 0.1, 24, and 48 hours. The oxygen content (% of saturation) was 92% at 0.1, 24, and 48 hours. The water temperature was 20.1, 20.2, and 20.2°C at 0.1, 24, and 48 hours, respectively.

Reference:	NATEC Institut (1997). Study Number NA 96 9410/3.3 "Acute Toxicity Test on the Orfe (*Leuciscus idus melanotus*) – Static Test Procedure, 48-Hours: BHMT-HP – Polyamine" (June 3).
Reliability:	Medium because a suboptimal study design was used. Only nominal concentrations were reported.

Type:	**96-hour LC_{50}**
Species:	Freshwater fish
Value:	79.6 mg/L
Method:	Modeled, ECOSAR (using log Kow of 1.80)
GLP:	Not Applicable
Test Substance:	BHMT
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates:

Type:	**48-hour EC_{50}**
Species:	Daphnid
Value:	5.7 mg/L
Method:	Modeled, ECOSAR (using log Kow of 1.80)
GLP:	Not Applicable
Test Substance:	BHMT
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants:

Type:	**96-hour EC_{50}**
Species:	Green algae
Value:	10.1 mg/L
Method:	Modeled, ECOSAR (using log Kow of 1.80)
GLP:	Not Applicable
Test Substance:	BHMT
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral ALD**
Species/Strain:	Male rats/Crl:CD®BR
Value:	1500 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. The test substance was suspended in corn oil and administered to 1 rat per dose level by intragastric intubation. Dose levels used in the study included 200, 450, 670, 1000, 1500, 2300, and 3400 mg/kg. Male rats were 7 weeks of age when received for the study and 8 weeks of age at test substance administration. Following administration of the test substance, rats were observed for clinical signs of toxicity. Surviving rats were weighed and observed daily until signs of toxicity subsided, and then at least 3 times a week throughout the 14-day observation period. Observations for mortality and signs of illness, injury, or abnormal behavior were made daily throughout the study. Pathological examinations were not performed.

GLP:	No
Test Substance:	Crude BHMT which consisted of: ~60% BHMT ~ 26% oligomeric amines ~5% C_{10} amines ~3% hexamethylenediamine ~5% caprolactam ~1% 6-aminocapronitrile On a dry-weight basis
Results:	The rats dosed at 2300 and 3400 mg/kg were found dead approximately 1 hour after dosing, and the rat dosed at 1500 mg/kg was found dead 1 day after dosing. Weight loss of approximately 5% of initial body weight was observed the day after dosing in the rat dosed at 1000 mg/kg. No other clinical signs of toxicity were reported.
Remark:	This estimate of the ALD varies from the LD50 determined in the following study; however, 1) the percent water in the different preparations was not documented, 2) the composition of the material was different and 3) one study used corn-oil gavage while the other dosed the material without dilution.
Reference:	DuPont Co. (1996). Unpublished Data, Haskell Laboratory Report No. 813-95, "Approximate Lethal Dose (ALD) in Rats" (February 8).
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	**Oral LD_{50}**
Species/Strain:	Male and female rats/Sprague Dawley
Value:	450 mg/kg (95% confidence interval, 415-485 mg/kg)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Crude BHMT was evaluated for acute oral toxicity in groups of 5 Sprague-Dawley albino rats (2 or 3/sex/group) administered single undiluted doses of 316, 398, 501, or 631 mg/kg by oral gavage. Clinical signs were periodically recorded throughout the study. Gross pathology was conducted on rats which died during the study and on survivors at study termination. The LD_{50} was calculated by the method of E. J. de Beer.
GLP:	No, predates GLP

(February 11, 2003)

Test Substance: Crude BHMT, purity not reported

Results: Mortality occurred within 1 to 2 hours in the 3 highest dose groups.

Dose (mg/kg)	Mortality	
	Males	Females
316	0/2	0/3
398	0/3	1/2
501	1/2	3/3
631	3/3	2/3

Clinical toxicity was characterized by reduced appetite, hypoactivity, rapid loss of vigor, collapse, and death. Upon necropsy of the decedents, hemorrhagic lungs and liver, and profound gastrointestinal inflammation were identified. The survivors of 7-day post-gavage observation exhibited no gross pathology attributable to treatment.

The oral LD_{50} was determined to be 450 mg/kg with limits of 415 to 485 mg/kg for combined sexes.

Remark: Although not specifically investigated, females appear to be the more sensitive sex based on this limited investigation. Also, see the remark for the ALD determination concerning the differences between the ALD and the LD50 studies.

Reference: Younger Laboratories Inc. (1973). Toxicological Investigation of: BHMT (bis-(Hexamethylene)-triamine), Monsanto Project Number Y-73-122 (July 16) (cited in TSCA Fiche OTS0534833).

Reliability: High because a scientifically defensible or guideline method was used. Although the group size was small, the close spacing of the dose levels increases confidence in the determination.

Additional References for Acute Oral Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 352-74, "Acute Oral Test" (June 3).

DuPont Co. (1961). Unpublished Data, Haskell Laboratory Report No. 38-61, "Acute Testing" (August 24).

Type:	**Inhalation Toxicity**
Species/Strain:	Rat/Albino
Value:	LC_{50} (6 hour) > vapor saturation at ambient temperature
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Six mature male albino rats were placed in a 35-L stainless steel chamber and exposed for six hours to an atmosphere containing vapors of the test substance. The atmosphere was generated by passing air at a rate of 4.0 L/min through a 500 mg flask containing 179.2 grams test substance at the beginning of the exposure. The method of passing the air through the test substance was such that the test substance was violently agitated by the air passing through. The atmosphere was passed through a one-liter bottle to remove droplets and then into the exposure chamber. No supplemental air was introduced into the exposure chamber. The animals were observed for behavior during the six-hour exposure period and for 10 days after exposure. At the end of this period, surviving animals were killed and examined macroscopically. During exposure the average temperature was 75°F with an 80% relative humidity inside the chamber.
GLP:	No, predates GLP
Test Substance:	Crude BHMT, purity not reported
Results:	No animal died during the exposure or 10-day observation period. During the exposure, rats were described as showing "slight lethargy" lasting up to one hour with no apparent weakness or drowsiness during the remainder of the exposure period. No nasal or ocular discharge was observed, and respiration appeared normal. Clinical observations and weight gains during the 10-day observation period were unremarkable. At necropsy the viscera appeared normal by macroscopic examination.
Remark:	The test substance was reported to be completely recovered in the flask at the end of the study. As this material has a low vapor pressure it is likely that the atmosphere was partially saturated by the more volatile components of the mixture and the lack of weight loss is accounted for by absorption of water and carbon dioxide by the test material. Based on the estimated vapor pressure range of 0.0003 to 0.0009 mm Hg (BHMT, EPIWIN) the likely actual exposure level at saturation ranged from 0.4 to 1.2 ppm. Based on the method employed, the actual exposure to BHMT is estimated to be approximately 0.75 ppm.

Reference:	Younger Laboratories Inc. (1973). "Toxicological Investigation of: BHMT (bis-(Hexamethylene)-triamine)", Monsanto Project Number Y-73-122 (July 16) (cited in TSCA Fiche OTS0534833).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity: None Found.

Type:	**Dermal ALD**
Species/Strain:	Rabbit/New Zealand White
Exposure Time:	24 hours
Value:	> 200 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. A single dose of the test substance was applied to the shaved, intact skin of 1 young adult male rabbit at a dosage of 200 mg/kg. The test substance was spread over a gauze pad which was then applied to the intact skin of the rabbit. The rabbit was then wrapped with successive layers of plastic film, stretch gauze bandage, and elastic adhesive bandage to form an occlusive dressing. The rabbit weighed 2553 g on the day of treatment. Approximately 24 hours after treatment, the wrappings were removed. Excess test substance was washed from the rabbit's back. The animal was weighed, observed for clinical signs of toxicity and dermal irritation, and returned to its cage. Observations for clinical signs of toxicity were made approximately 3 hours after dosing and then daily thereafter for 14 days after treatment (1 weekend excluded). Observations for mortality were made daily throughout the study. The rabbit was weighed on days 1, 4, 7, 13, and 14 following treatment. No pathology was conducted. Dermal effects were scored according to the Draize scale.
GLP:	Yes
Test Substance:	Crude BHMT which consisted of: 50-60% BHMT 3-5% Caprolactam 1-3% 6-Aminocapronitrile 1-3% 6-Aminocaproamide 0-5% Decanediamines

	0-3% Hexamethylenediamine 6-50% Other amines and nitriles
Results:	The rabbit survived the 14-day observation period. No clinical signs of toxicity were observed during the study. The rabbit lost approximately 3% of its body weight from day 13 to day 14 after application. However, the rabbit gained weight over the duration of the study. Necrosis, mild to severe erythema, and mild to severe edema were observed throughout the study. Green discoloration, cratering, sloughing of the epidermis, and raw areas were observed at the test site. Additional rabbits were not treated because of the corrosive nature of the test substance.
Reference:	DuPont Co. (1993). Unpublished Data, Haskell Laboratory Report No. 564-93, "Approximate Lethal Dose (ALD) by Skin Absorption of BHMT Amine in Rabbits" (October 25).
Reliability:	High because a scientifically defensible or guideline method was used.
Type:	**Dermal ALD**
Species/Strain:	Male and female rabbits/New Zealand White
Exposure Time:	24-hours
Value:	> 126 and < 200 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. BHMT was evaluated for acute dermal toxicity in alternate solitary male or female New Zealand white rabbits administered single occluded applications of 31.6, 50.1, 79.4, 126, 200, 316, 1000, and 2000 mg/kg neat test substance upon clipped, intact skin for 24 hours. Animals were observed for 14 days post-dosing, after which the survivors were sacrificed and examined macroscopically.
GLP:	No, predates GLP
Test Substance:	Crude BHMT, purity not reported
Results:	An LD_{50} range was identified between 126 and 200 mg/kg, with each rabbit receiving a 200 mg/kg dose or higher succumbing from several hours to one day post-treatment. Animals at lower doses survived the 14-day observation period. Reduced appetite*, hypoactivity*, increasing loss of vigor, collapse, and death characterized the clinical evidence of systemic toxicity. Upon necropsy, macroscopic evidence of systemic toxicity associated with treatment was identified

among the decedent rabbits, including hemorrhagic lungs and liver. The study survivors of 14-day post-treatment observation exhibited no gross lesions.

* Also noted in surviving animals, lasting up to one day.

Reference:	Younger Laboratories Inc. (1973). "Toxicological Investigation of: BHMT (bis-(Hexamethylene)-triamine)", Monsanto Project Number Y-73-122 (July 16) (cited in TSCA Fiche OTS0534833).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Dermal Toxicity: None Found.

Type:	**Dermal Irritation**
Species/Strain:	Rabbit/New Zealand White
Exposure Time:	24 hours
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. A single dose of the test substance was applied to the shaved, intact skin of 1 young adult male rabbit at a dosage of 200 mg/kg. The test substance was spread over a gauze pad, which was then applied to the intact skin of the rabbit. The rabbit was then wrapped with successive layers of plastic film, stretch gauze bandage, and elastic adhesive bandage to form an occlusive dressing. The rabbit weighed 2553 g on the day of treatment. Approximately 24 hours after treatment, the wrappings were removed. Excess test substance was washed from the rabbit's back. The animal was weighed, observed for clinical signs of toxicity and dermal irritation, and returned to its cage. Observations for clinical signs of toxicity were made approximately 3 hours after dosing and then daily thereafter for 14 days after treatment (1 weekend excluded). Observations for mortality were made daily throughout the study. The rabbit was weighed on days 1, 4, 7, 13, and 14 following treatment. No pathology was conducted. Dermal effects were scored according to the Draize scale.
GLP:	Yes
Test Substance:	Crude BHMT which consisted of: 50-60% BHMT

	3-5% Caprolactam 1-3% 6-Aminocapronitrile 1-3% 6-Aminocaproamide 0-5% Decanediamines 0-3% Hexamethylenediamine 6-50% Other amines and nitriles
Results:	The rabbit survived the 14-day observation period. Necrosis, mild to severe erythema, and mild to severe edema were observed throughout the study. Green discoloration, cratering, sloughing of the epidermis, and raw areas were observed on the test site. The material was considered corrosive to rabbit skin after 24-hour exposure. Additional rabbits were not treated because of the corrosive nature of the test substance.
Reference:	DuPont Co. (1993). Unpublished Data, Haskell Laboratory Report No. 564-93, "Approximate Lethal Dose (ALD) by Skin Absorption of BHMT Amine in Rabbits" (October 25).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Dermal Irritation:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Rhone Poulenc, Inc. (1981). Bioassay Systems Corporation Project Number 10580, "DOT Rabbit Skin Corrosion Test and STEEL Corrosion Test" (May 20) (cited in TSCA Fiche OTS0543734).

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 181-74, "Department of Transportation Skin Corrosion Test on Rabbit Skin" (March 5).

Monsanto Co. (1972). "Skin Irritation in Rabbits After Application of: Bishexylmethylenetriamine (50% Concentration)" (March 23) (cited in TSCA Fiche OTS0538588).

Type:	**Dermal Sensitization:** No Data.

Type:	**Eye Irritation**
Species/Strain:	Rabbits/Albino
Exposure Times:	1 minute and 24 hours

Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Crude BHMT was evaluated for eye irritation in 2 groups of 3 (2 male, 1 female) albino rabbits administered single 0.1 mL undiluted instillations into right eye conjunctival sacs for 1 minute and 24 hours, respectively. Treatment was terminated by saline wash of the treated eyes. Determination of irritation at 10 minutes, 1 hour, and at 3, 5, 7, and 14 days post-treatment were made based on the method of Draize, Woodard, and Calvery. The left (untreated) eyes served as control.
GLP:	No, predates GLP
Test Substance:	Crude BHMT, purity not reported
Results:	One hour after rinse of the 1-minute instillation, the initial severe erythema, slight edema, and copious discharge had worsened to severe erythema, moderate edema, copious discharge, and corneal dullness. Rabbits in the 24-hour treatment group exhibited a severe irritation response by 10 minutes. Increasing corneal cloudiness in the animals of 1-minute instillations progressed to corneal opacity at 120 hours. Corneal opacity was reported 1 hour after instillation for the eyes that were not rinsed after 1 minute. The material is considered corrosive to the eye.
Reference:	Younger Laboratories Inc. (1973). "Toxicological Investigation of: BHMT (bis-(Hexamethylene)-triamine)" (July 16), Monsanto Project Number Y-73-122 (cited in TSCA Fiche OTS0534833).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Eye Irritation: None Found.

5.2 Repeated Dose Toxicity:

Type:	**13-Week Oral Study**
Species/Strain:	Rats/Sprague Dawley
Sex/Number:	Male and female/15 per sex per group
Exposure Period:	13 weeks
Frequency of Treatment:	Daily
Route:	Oral gavage in water
Exposure Levels:	0, 20, 50, 120 mg/kg/day
Method:	No specific test guideline was reported; however, a

scientifically defensible approach was used to conduct the study.

Dosing solutions were analyzed at weeks 1, 4, 8, and 12, and were generally within ±10% of target concentrations. Stability of the dosing solutions was verified after storage for 1 and 10 days at room temperature.

Crude BHMT was administered by oral gavage in a deionized water vehicle to groups of 15 Charles River CD® rats of each sex. Rats were about 28 days old when received and were acclimated for 2 weeks prior to study start.

The rats were checked twice daily for moribundity or mortality. Weekly detailed physical examinations were performed. Body weights and food consumption were measured weekly throughout the study. Pretest and week 13 eye examinations were conducted.

Clinical laboratory evaluations were performed on 10 rats/sex/group at week 13. Ten hematology parameters and 20 blood chemistry parameters were measured or calculated. Urinalysis examinations included dipstick examination of 9 parameters and microscopic examination of 4 parameters.

Rats sacrificed as moribund and those surviving to terminal sacrifice were examined for gross lesions at necropsy. Tissue masses and gross lesions at all dose levels were retained for microscopic examination. Approximately 39 tissues were removed from all dose groups. The tissues from the high and control groups were prepared and examined histologically. For the low- and mid-dose groups, the liver, kidneys, and lungs were sectioned for microscopic analysis. Brain, kidneys, adrenals, heart, liver, and ovaries or testes of all groups were weighed.

Body weight (weekly), food consumption (weekly), clinical laboratory (week 13) and organ weight (absolute and relative to body and brain weights, terminal sacrifice) data were analyzed using Bartlett's test for homogeneity of variance and analysis of variance (one way classification). Treatment groups were compared to the control group, by sex, using the appropriate t-statistic (equal or unequal variance), as described by Steel and Torrie, 1960 and Ostle, 1954. Dunnett's multiple comparison tables (Dunnett, 1964) were used to determine significance. Total bilirubin, gamma glutamyl transpeptidase, ornithine carbamoyltransferase, chloride and specific gravity were analyzed using a nonparametric approach, by transforming the data to ranks prior to analysis, as described by Conover and Imaa, 1981. All statistical tests were two-tailed, with $p<0.05$ and $p<0.01$ used as levels of significance.

Steel, R. G. D. and Torrie, J. H. (1960). Principles and Procedures of Statistics, McGraw-Hill Book Company, Inc., New York.

Ostle, B. (1954). Statistics in Research, Iowa State College Press, Ames, Iowa.

Dunnett, C. R. (1964). Biometrics, 20:482-491.

Conover, W. J. and Iman R. L. (1981). The American Statistician, 35:124-133.

GLP: Yes

Test Substance: Crude BHMT. The test material was described as a black paste containing 65% BHMT, 32% tars, 2% adiponitrile, and 1% aminocapronitrile (on a dry basis).

Results: One male and 1 female in the high-dose group died and 1 male at the mid-dose group died during the treatment period, but this mid-dose death was not considered to be treatment related. With the possible exception of respiratory rales seen in a few males and females at the high-dose group and mid-dose females, no clinical signs of toxicity associated with treatment were observed.

The high-dose females showed statistically decreased body weights in comparison to controls in weeks 6 and 7, and at other times. At study end there were no statistically significant differences. Terminal body weights are shown in the table below. No significant weight differences were measured in males at any dose level or females at the low- and mid-dose levels. Some evidence of reduced food consumption was noted for high-dose males.

Dose (mg/kg)	Group Mean Body Weights at Termination (g)	
	Males	Females
0	504	281
20	491	286
50	494	276
120	498	267*

* Statistically different from control.

Ophthalmoscopic examination did not reveal any effects of treatment on the eye.

With the exception of an increase in segmented neutrophils in high-dose males and females, there was no evidence of treatment-related changes in the hematolgic, clinical chemistry, or urinalysis examinations.

The incidental findings from gross necropsy did not appear to be treatment related. Males at the high-dose level had decreased absolute adrenal weights, and the liver weight relative to body weight for mid-dose females was elevated. These changes were not correlated with any microscopic findings and were considered unrelated to treatment. No other significant organ weight differences between control and treatment groups were noted. The microscopic lesions observed were generally comparable in treated and control group animals and were considered to be of spontaneous origin. Interstitial pneumonia was slightly increased in treatment groups, but was considered non-specific and unrelated to treatment. Therefore, treatment did not result in histopathologic changes in any organ or tissue.

The no-observable adverse effect level (NOEL) was considered to 50 mg/kg/day for rats of each sex by the study authors.

Reference: International Research and Development Company sponsored by Monsanto Co. (1985). Report No. IR-83-154, "Thirteen-Week Oral Study in Rats" (March 12).

Reliability: High because a scientifically defensible or guideline method was used.

Type: **13-Week Inhalation Study**

Species/Strain: Rat/Sprague Dawley

Sex/Number:	Male and female/12 per sex per group
Exposure Period:	13 weeks (total of 65 exposures)
Frequency of Treatment:	6 hours/day, five days/week
Exposure Levels:	0, 10, 30, 60 mg/m^3
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Inhalation exposures were conducted in 1.75 m^3 Rochester chambers by nebulization aerosol generation. The test material, diluted in water was delivered to the nebulizer using a Harvard Infusion Pump. Atmospheres passed through a vertical particle size separator for the low and mid-concentrations chambers, but were admitted directly into the chamber at the high concentration. The air flow rate was maintained at a constant flow of 340 L/min. Animals were individually housed in the chambers and their positions were rotated weekly. Charles River Sprague-Dawley rats were 54 days old on the first day of the study, males weighed 227-237 grams and females weighed 136-199 grams. Food and water was withheld during the exposures.

Test material concentrations in air were measured 3 to 4 times daily for each treatment chamber by collection on impingers containing 2-propanol. Analytical determinations of BHMT in 2-propanol samples were done by gas chromatography. Chamber distribution measurements were also performed at periodic intervals. Particle size distribution was determined gravimetrically using an Anderson Impactor twice weekly during the first 2 weeks and weekly thereafter.

All rats were observed prior to and after exposure and twice daily during exposure. Detailed physical examinations and body weights were recorded weekly. Ophthalmic examinations were conducted on all rats pre-exposure and on the control and high-dose rats in week 12.

Clinical laboratory evaluations were performed on 5 rats/sex/group at termination. Ten hematology parameters and 11 blood chemistry parameters were measured or calculated.

At gross necropsy examinations, tissue masses and gross lesions at all dose levels were retained for microscopic examination. Approximately 36 different organs/tissues were removed from all dose groups. The tissues from the high and control groups were prepared and examined histologically. For the low- and mid-dose groups, the nasal passages, trachea, and lungs were sectioned for microscopic analysis. Brain, kidneys, adrenals, heart, spleen, liver, and testes with epididymides of all groups were weighed.

Data were analyzed by parametric or non-parametric techniques to assess the statistical significance between control and treatment groups. Body weights and absolute organ weights were evaluated by Analysis of Variance (Fisher, 1946) and Dunnett's (two tailed) Test (Dunnett, 1955) to detect significant differences between means of treated groups and those of the respective control group. Clinical chemistry data were analyzed with Dunnett's Test only. Bartlett's Test (Bartlett, 1937) was used to assess the variability of these data. Statistical analyses of organ-to-body weight ratios were performed using the Mann-Whitney Test (Siegel, 1956) with Bonferroni's Inequality Procedure (Ryan, 1959; Miller, 1966). Differences in the frequency of lesions were determined using Fisher's exact test (Ryan, 1959) with Bonferroni's Inequality Procedure.

Fisher, R. A. (1946). Statistical Methods for Research Workers, Oliver and Boyd, Edinburgh.

Dunnett, C. W. (1955). Jour. Am. Stats. Assoc., 1096-1121.

Bartlett, M. S. (1937). Supplement to the Journal of the Royal Statistical Society, 4:137.

Siegel, S. (1956). Nonparametric Statistics for the Behavioral Sciences, McGraw Hill Co., NY.

Ryan, T. A. (1959). Psychol. Psychological Bull, 56:25-47.

Miller, R. G. (1966). Simultaneous Statistical Inferences, McGraw Hill Co., NY.

GLP: Yes

Test Substance: Crude BHMT. The test material identification sheet described the test material as CAS NO 68411-90-5, BHMT Mixture. It was stated to contain 48.7% water and, on a dry basis, 63.26% BHMT, 7.35% hexamethylenediamine, ~2% adiponitrile, and 25-30% unknowns. The source was listed as "holding tank 6/85".

Results:

Mean analytical values over the entire course of the study for the 3 exposure levels were 10, 31, and 62 mg/m^3. The nominal to analytical ratios ranged from 3.2 to 5.9 which resulted from substantial deposition of the test material on chamber surfaces; however, chamber distribution data indicated acceptable dispersion of the aerosol. Generally, greater than 90% of the test material particles were in the respirable range. Test material was shown to be stable.

No animals died on study. Clinical signs of toxicity were confined to respiratory wheezing in high dose animals and discoloration of fur in females. High dose animals exhibited significantly decreased body weight in comparison to control animals.

Elevated red blood cell counts with associated increases in hemoglobin and hematocrit were observed in high dose males (9%) and females (12%). Alanine aminotransferase, aspartate aminotransferase, and phosphorous levels were elevated in high dose males (primarily attributed to changes in a few animals), but there were no histopathological correlates. High dose females had lowered serum glucose levels that were considered treatment related, but biologically insignificant. Other differences in clinical chemistry seen at the low and mid-dose level were within normal ranges and were not considered treatment related.

In high-dose animals, several organs showed reduced absolute weights and increased relative weights, which were attributed to overall body weight reductions and not specific organ effects. Dose-related increases in the incidence of pulmonary emphysema were noted at gross necropsy.

Lesions associated with treatment were restricted to the respiratory tract. Three target organs were affected: the nasal passages, trachea, and lungs all showed graduated progression of responses from hypertrophy to squamous metaplasia hyperplasia to ulceration of the ciliated respiratory epithelium. The incidence and severity of the lesions was increased as a function of higher exposure levels, being most notable in the 2 highest dose groups and much less severe at the low dose level.

Hypertrophy and eosinophilic granularity of the olfactory epithelium was noted at all levels of treatment and was considered to be an age-related change exacerbated by treatment. Hypertrophy, hyperplasia, squamous metaplasia, and ulceration were found in the nasal passages, trachea, and lungs with secondary inflammation of the ciliary epithelium. These responses were consistent with those expected for a moderate irritant; there was no evidence that these changes represented a pre-neoplastic condition.

The pulmonary emphysema noted at gross necropsy was related to the histopathological changes and may have resulted in hypoxia and associated increases in red blood cell production.

A no-effect level was not determined in the study.

Reference:	Monsanto Co. (1987). Report No. ML-85-220, "BHMT Thirteen-Week Inhalation Study" (March 19) (also cited in TSCA Fiche OTS0545432).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Repeated Dose Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Monsanto Co. (1983). Report No. IR-83-152; 401-220, "Four-Week Oral Rangefinding Study in Rats" (December 13).

Monsanto Co. (1963). Report No. ML-85-109, "Two-Week Inhalation Toxicity Study" (December 1).

5.3 Developmental Toxicity:

Species/Strain:	Rats/Charles River CD®
Sex/Number:	Female/25
Route of Administration:	Gavage
Exposure Period:	Days 6-15 of gestation
Frequency of Treatment:	Daily
Exposure Levels:	0, 50, 100, 250 mg/kg/day
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Crude BHMT (3 dose levels plus control) was administered to groups of 25-mated rats. Females were Charles River COBS CD rats received at about 70 days of age. After a 15-day acclimation period females were bred to males of the same strain from the same source, mating a single female with one male. When a copulatory plug was observed the female was considered pregnant and it was designated day 0 of gestation. Females weighed between 213 and 274 g on gestation day 0. Before and after mating, females were individually housed in a suspended wire-mesh cage. Dosages were prepared in water vehicle at a concentration of 0.2 grams/mL of solution. Animals were observed twice daily for mortality or signs of toxicity. Detailed physical examinations were performed on gestations days 6 through 20. Maternal body weights were recorded on days 0, 6, 9, 12, 16, and 20.

All surviving rats were sacrificed at day 20 of gestation. A gross postmortem examination was performed on all animals. The numbers and location of live and dead fetuses, early and late resorptions, implantation sites, and corpora lutea were recorded. Fetuses were weighed, sexed, and examined for gross and external malformations. Half of the fetuses were sectioned and examined for soft tissue malformations. The remaining half were processed by alizarin red staining for subsequent skeletal examination.

All statistical analyses compared the treatment groups to the control with the level of significance at $p<0.05$ and $p<0.01$. Male to female fetal sex ratios and the proportions of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described by Siegel, 1956 to judge significance of differences. The proportions of resorbed and dead fetuses and post-implantation losses were compared by the Mann-Whitney U-test as described by Siegel, 1956 and Weil, 1970 to judge significance of differences. Mean numbers of corpora lutea, total implantations, post-implantation loss, live fetuses, and mean fetal body weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances, and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie, 1960 using Dunnett's multiple comparison tables (Dunnett, 1964) to judge significance of differences.

Siegel, S. (1956). Nonparametric Statistics for the

Behavioral Sciences, McGraw-Hill, New York, NY.

Weil, C. S. (1970). Food Cosmet. Toxicol., 8:177-182.

Steel, R. G. D. and Torrie, J. H. (1960). Principles and Procedures of Statistics, McGraw-Hill, New York, NY.

Dunnett, C. W. (1964). Biometrics, 20:482-491.

GLP: Yes

Test Substance: BHMT Unrefined, containing 18.9% water

Results: No early deaths occurred in the control group. In the low, mid and high dose groups, 1, 6, and 5 dams, respectively, died or were sacrificed as moribund. No particular cause of death was observed at necropsy in these rats. Rats in all treatment groups exhibited increases in respiratory difficulty as compared to controls. Slight reductions in body weights were seen in all treatment groups beginning at the first dosing day. The treatment groups continued to lag behind control body weights through termination, but the overall reductions in weight gain were not dose-related. In spite of a high variability in body weight gains the data suggest the there was no dose-related body weight changes.

The necropsy findings in the surviving dams did not reveal any treatment-related increase in macroscopic lesions.

No treatment-related effects on fetal ossification variations, external, soft tissue, or skeletal malformations were found. Decreases in mean numbers of corpora lutea and total implantations, and increases in post-implantation losses resulted in decreased numbers of viable fetuses in the 50 and 100 mg/kg/day dose groups. No notable differences were seen in these parameters for the 250 mg/kg/day group in comparison with controls. Since there was no effect at the high dose and no dose-related trend (the 50 mg/kg/day appeared to be marginally more affected than the 100 mg/kg/day group), the findings were not considered to be treatment related.

A summary of some of the reproductive outcomes (means/litter) are provided in the tables below:

Dosage (mg/kg):	0	50	100	250
N:	25	24	19	20
Corpora lutea:	16.3	15.4	15.8	16.7
Implantations:	15.3	14.2	14.9	15.4
Postimplantation Loss:	0.6	1.5	1.9	0.9
Total No. of Fetuses:	14.7	12.6	13.0	14.5
Total No. of Live Fetuses:	3.2	3.3	3.1	3.3
Mean Fetal Weight (g):	47.8	50.2	42.9	54.4
Sex Ratio (No. of Males/Total No.):	16.3	15.4	15.8	16.7

BHMT did not appear to be embryotoxic, fetotoxic, or teratogenic.

Fetal NOEL: 250 mg/kg
Maternal NOEL: none determined (> 250 mg/kg-bw)

Reference: International Research and Development Company sponsored by Monsanto Co. (1985). Report No. IR-83-106, "BHMT Teratology Study" (March 12).

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Developmental Toxicity: None Found.

5.4 Reproductive Toxicity:

Study No. 1: 13-Week Oral Study

Species/Strain: Rats/Sprague Dawley
Sex/Number: Male and female/15 per sex per group
Route of Administration: Gavage
Exposure Period: 13 weeks

Frequency of Treatment:	Daily
Exposure Levels:	0, 20, 50, 120 mg/kg/day
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Crude BHMT was administered by oral gavage in a deionized water vehicle to groups of 15 Charles River CD® rats of each sex. Rats were about 28 days old when received and were acclimated for 2 weeks prior to study start. The stability of the test article in vehicle was demonstrated analytically and the test article dilutions were analyzed in weeks 1, 4, 8, and 12. The rats were checked twice daily for moribundity or mortality. Weekly detailed physical examinations were performed. Body weights and food consumption were measured weekly throughout the study. Rats sacrificed as moribund and those surviving to terminal sacrifice were examined for gross lesions at necropsy. Tissue masses and gross lesions at all dose levels were retained for microscopic examination. Additional details regarding the subchronic toxicity endpoints evaluated can be found in Section 5.2. Reproductive endpoints evaluated in this test included the following. Ovaries, testis with epididymis, mammary region, and uterus were removed from all applicable dose groups. The tissues from the high and control groups were prepared and examined histologically. Ovaries or testes of all groups were weighed.
GLP:	Yes
Test Substance:	Crude BHMT. The test material was described as a black paste containing 65% BHMT, 32% tars, 2% adiponitrile, and 1% aminocapronitrile (on a dry basis)
Results:	One male and 1 female in the high-dose group died and 1 male at the mid-dose group died (mid-dose death not considered related to treatment) during the treatment period. The high-dose females showed decreased body weights in comparison to controls. No significant weight differences were measured in males at any dose level or females at the low- and mid-dose levels. The incidental findings from gross necropsy did not appear to be related to treatment. No significant organ weight differences between control and treatment groups were noted

	in the reproductive organs. Treatment did not result in histopathologic changes in any reproductive organ. Additional details regarding subchronic toxicity results can be found in Section 5.2.
Reference:	International Research and Development Company sponsored by Monsanto Co. (1985). Report No. IR-83-154, "Thirteen-Week Oral Study in Rats" (March 12).
Reliability:	High because a scientifically defensible or guideline method was used.

Study No. 2: 13-Week Inhalation Study

Species/Strain:	Rat/Sprague Dawley
Sex/Number:	Male and female/12 per sex per group
Route of Administration:	Inhalation
Exposure Period:	13 weeks (total of 65 exposures)
Frequency of Treatment:	6 hours/day, five days/week
Exposure Levels:	0, 10, 30, 60 mg/m^3
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. All rats were observed prior to and after exposure and twice daily during exposure. Detailed physical examinations and body weights were recorded weekly. At gross necropsy examinations, tissue masses and gross lesions at all dose levels were retained for microscopic examination. Additional details regarding the subchronic toxicity endpoints evaluated and details of the methodology can be found in Section 5.2. Reproductive endpoints evaluated in this test included the following. Ovaries, testis with epididymis, mammary gland, vagina, and uterus were removed from all applicable dose groups. The tissues from the high and control groups were prepared and examined histologically. Testes with epididymides of all male groups were weighed.
GLP:	Yes
Test Substance:	Crude BHMT. The test material identification sheet described the test material as CASNO 68411-90-5, BHMT Mixture. It was stated to contain 48.7% water and, on a dry basis, 63.26% BHMT, 7.35% hexamethylenediamine, ~2% adiponitrile, and 25-30% unknowns. The source was listed as "holding tank 6/85".

Results:	No animals died on study. Clinical signs of toxicity were confined to respiratory wheezing in high dose animals and discoloration of fur in females. High dose animals exhibited significantly decreased body weight in comparison to control animals. In high-dose animals, several organs showed reduced absolute weights and increased relative weights, which were attributed to overall body weight reductions. No significant organ weight differences between control and treatment groups were noted in the reproductive organs. Treatment did not result in histopathologic changes in any reproductive organ. Additional details regarding subchronic toxicity results can be found in Section 5.2.
Reference:	Monsanto Co. (1987). Report No. ML-85-220, "BHMT Thirteen-Week Inhalation Study" (March 19) (also cited in TSCA Fiche OTS0545432).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Reproductive Toxicity: None Found.

5.5 Genetic Toxicity

Type:	***In vitro* Bacterial Reverse Mutation Assay**
Tester Strain:	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 *Escherichia coli* strain WP2 *uvr*A
Exogenous Metabolic Activation:	With and without Aroclor-induced rat liver S-9 homogenate
Exposure Concentrations:	Experiment A: 0, 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, 5000 μg/plate Experiment B1: 0, 25, 75, 200, 600, 1800, 5000 μg/plate Experiment B2: 0, 25, 75, 200, 600, 1800, 2500, 5000 μg/plate Experiments B3 and B4: 0, 25, 75, 200, 600, 1800, 2500, 3333, 5000 μg/plate
Method:	The procedure used in the test was based on the recommendations of the following guideline: OECD Guideline 471.

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In Experiment A (the preliminary toxicity test), the vehicle (dimethyl sulfoxide, DMSO) and ten dose levels of the test substance were plated, 1 plate per dose, with overnight cultures of TA98, TA100, TA1535, TA1537, and WP2 *uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9. The test was conducted to establish the dose range over which the test substance would be tested.

In the mutagenicity tests (Experiments B1-B4), the test system was exposed to the test substance via the plate incorporation method originally described by Ames, B. N. et al. (1975). Mutat. Res., 31:347-364 and updated by Maron, D. M. and B. N. Ames (1983). Mutat. Res., 113:173-215.

DMSO was initially selected as the solvent of choice based on compatibility with the target cells and solubility of the test substance. It was used as the solvent in the preliminary toxicity test. With the exception of a few tiny black particles, the test substance was soluble and clear in DMSO at a maximum concentration of 250 mg/mL. While DMSO would allow the bacterial mutation test to be conducted at the regulatory required top dose of 5 mg/plate, the regulatory required top doses could not be achieved in the other genetic toxicity assays using DMSO. For this reason, ethanol was subsequently selected as the solvent of choice based on compatibility with the target cells and solubility of the test substance. The test substance was soluble and clear in ethanol at approximately 500 mg/mL, the maximum concentration tested.

Positive control substances used in the test included 2-aminoanthracene, methyl methanesulfonate, 9-aminoacridine, sodium azide, and 2-nitrofluorene.

All dose levels of the test substance, vehicle controls (ethanol), and positive controls were plated in triplicate. S9 or sham mix, tester strain and vehicle, positive control, or test substance dilution were added to top agar. After vortexing, the mixture was overlaid onto the surface of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37±2°C. Plates that were not counted immediately following the incubation period were stored at 2-8°C until colony counting could be conducted (< 10 days).

The metabolic activation system involved the addition of S9 mixture. The S9 mixture contained S9, glucose 6-phosphate, β-nicotinamide-adenine dinucleotide phosphate, $MgCl_2$, and KCl in a phosphate buffer. The sham S9 mix contained phosphate buffer.

The condition of the background lawn was evaluated for evidence of test substance toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Revertant colonies for a given test strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.

For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated. For the test substance to be evaluated positive, it must have caused a dose-related increase in the mean revertants per plate of at least 1 tester strain over a minimum of 2 increasing concentrations of the test substance. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3 times the mean vehicle control value. Data sets for tester strains TA98, TA100, and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2 times the mean vehicle control value.

GLP: Yes

Test Substance: Crude BHMT which consisted of:

46% Bis(hexamethylene)triamine
54% Other oligomeric amines

Results: Negative

Remarks: In the preliminary toxicity test (Experiment A), toxicity was observed at 3333 and 5000 μg/plate. No precipitate was observed.

In Experiment B1, no positive responses were observed with tester strains TA98, TA1535, TA1537, and WP2 *uvr*A in the presence of S9 activation and with any of the tester strains in the absence of S9 activation. No precipitate was observed, but toxicity was observed beginning at 1800 or at 5000 μg/plate. Tester strain TA100 in the presence of S9 was retested in Experiment B2 due to an unacceptable

	positive control value. A 2.4-fold, non-dose responsive increase was observed with tester strain TA1537 in the presence of S9 activation. Tester strain TA1537 was also retested in Experiment B2 to clarify this response.
	In Experiment B2, no positive responses were observed with tester strains TA100 and TA1537 in the presence of S9 activation. No precipitate was observed, but toxicity was observed at 2500 and 5000 µg/plate. A 2.9-fold, non-dose responsive increase was observed with tester strain TA1537 in the presence of S9 activation. Tester strain TA1537 was retested in Experiments B3 and B4 using 5% and 15% S9 mix.
	In Experiments B3 and B4, no positive responses were observed with tester strain TA1537 in the presence of 5% and 15% S9 activation. No precipitate was observed, but toxicity was observed at 3333 and 5000 µg/plate.
Reference:	DuPont Co. (2002). Unpublished Data, Haskell Laboratory Report No. DuPont-10197, "Bacterial Reverse Mutation Test" (May 30).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Bacterial Reverse Mutation Assay:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Monsanto Co. (1985). Report No. HL-84-228, "Ames/*Salmonella* Mutagenicity Assay" (January 14).

Type:	***In vitro* Hepatocyte DNS Repair (UDS) Assay**
Tester Strain:	Primary rat hepatocytes
Exogenous Metabolic Activation:	Not Applicable
Exposure Concentrations:	0.1, 0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, 5000 µg/mL (Preliminary assay) 0, 10, 50, 100, 500, 750 µg/mL (Replicate assay)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.
	Primary hepatocytes were isolated from freshly excised

livers of adult male Fischer-344 rats. The cells were allowed to attach to glass cover slips in culture media supplemented with bovine serum for 1.5-2.0 hours. The non-viable cells were washed out and subsequent exposures were performed on viable hepatocytes in serum-free media.

For the preliminary assay, crude BHMT was diluted in culture medium and added to triplicate cultures at concentrations of 1, 5, 10, 50, 100, 500, 1000 and 5000 µg/mL. Cytotoxicity was observed at 1000 and 5000 µg/mL. Levels of 10, 50, 100, 500, and 750 µg/mL of the test material were selected for the definitive replicate assay. In addition to the test material dilutions, concurrent culture medium and 2-acetyl aminofluorene (2-AAF, 0.2 µg/ml) as negative and positive controls, respectively, were incubated with hepatocyte cultures for each assay.

Triplicate cultures at each level were exposed simultaneously to the test material and to ^{3}H-thymidine for 18 to 20 hours. After slide preparation, the slides were dipped in Kodak NTB-2 emulsion and exposed at -20°C for 7-12 days prior to development. Following staining with methyl-green Pyronin, nuclear and cytoplasmic tritium-exposed silver grains on the autoradiographic films were counted using an automated grain counter interfaced with a computer. The net nuclear grain count was determined by subtracting the background of the highest count of two nuclear size areas of the cytoplasm from the nuclear grain count. A minimum of 50 cells from each of 3 replicate slides were scored for each level of control and treatment groups. Test results were considered positive if the net nuclear grain count and percent of cells in repair were markedly greater than the concurrent negative control or greater than +5 net nuclear grains.

GLP: Yes

Test Substance: Crude BHMT. The test material was described as a black solid containing 65% BHMT, 32% tars, 2% adiponitrile, and 1% aminocapronitrile (on a dry basis)

Results: Negative

Remarks: The average net nuclear grain counts and percentage of cells in repair for the preliminary and replicate assay with controls and crude BHMT-treated groups did not demonstrate treatment-related genotoxicity.

Cytotoxicity was observed at 1000 and 5000 µg/mL in the preliminary assay. The net grain counts were similarly

	negative for both the medium control and all crude BHMT treatment levels in both assays. With the exception of a slight increase in the percent of cells in repair at 500 µg/mL in the replicate assay, negative control and test material treatment groups had comparable low percentages of cells in repair. Both assays gave a strong positive response to the positive control, which confirms the responsiveness of the cells cultured. Only visual examination of cells treated with the test material at 0.1 and 0.5 µg/mL was performed since higher treatment levels had not produced significant unscheduled DNA synthesis. This examination confirmed the absence of UDS at these lower levels.
Reference:	Monsanto Co. (1985). Report No. SR-84-305, SRI Project No. LSC-7795, "*In vitro* Hepatocyte DNA Repair (UDS) Assay" (April 3).
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	***In vitro* CHO/HGPRT Cell Gene Mutation Assay**
Tester Strain:	Chinese hamster ovary (CHO) cells
Exogenous Metabolic Activation:	Liver S9
Exposure Concentrations:	0, 10, 50, 100, 175, 250 µg/mL (without metabolic activation) 0, 1, 10, 100, 500, 1000 µg/mL (with metabolic activation)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Crude BHMT was tested for mutagenic activity at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene locus using CHO cells in culture. The toxicity of crude BHMT to CHO cells in the presence and absence of metabolic activation was determined in cytotoxicity studies prior to the selection of the concentrations for the mutagenicity assays. Preliminary mutation induction assays were conducted to determine additional toxicity information and optimal liver homogenate (S9) concentration, and to obtain an initial estimate of mutagenic potential. Based on the results of these rangefinding assays, optimal S9 concentrations for mutation induction and dose levels estimated to result in cytotoxicity of 10-100% were selected for the definitive mutation assays.

The definitive mutation induction assays were conducted on triplicate treatment samples at each of 5 concentrations with and without S9 metabolic activation. CHO cells were exposed to test or control compounds for 5 hours. Dimethylnitrosamine (DMN) and ethylmethanesulfonate (EMS) were the positive control agents in the presence and absence of S9 metabolic activation. Solvent and media negative controls were also evaluated with each assay.

After removal of the media containing the test material, the cells were incubated an additional 19 hours and then replated to determine toxicity and allow for the expression of mutations.

After the 7-day expression period, the cells were incubated in media containing 6-TG, the selective agent, for 7 days. Plating efficiency (survival) of the cells was concurrently determined by plating 200 cells/plate in media without 6-TG.

A confirmatory assay using the same concentrations and methodology as the initial assay was performed to confirm the results.

Statistical analysis of the data was based on methods published by Snee and Irr (1981). Mutat. Res., 85:77-93. The analysis consisted of transformation of the data followed by an analysis for linear trend (dose-response) and a pair-wise comparison of control and treatment mutant frequencies by a modified Student's t-test.

GLP: Yes

Test Substance: Crude BHMT, purity not reported, described as a "black waxy material"

Results: Negative

Remarks: In the cytotoxicity assay, increased concentrations of S9 mixture reduced the cytotoxicity of crude BHMT at similar dose levels.

Based upon results of the cytotoxicity assays, the initial mutagenicity assay was conducted at test material concentrations of 15, 150, or 300 μg/mL of treatment volume without metabolic activation or at dose levels ranging from 15 to 1500 μg/mL with 1, 2, 5 or 10% (v/v) concentrations of the S9 metabolic activation preparation. Analysis of the results indicated no significant increase in mutation frequency as compared to negative controls at any treatment concentration and no dose-response relationship.

The 5% concentration of S9 was chosen for the definitive mutation assay as this concentration tended to give moderate reductions in cytotoxicity.

Based upon the preliminary mutagenicity results, the definitive mutation assay was conducted at test material concentrations of 10, 50, 100, 175, or 250 µg/mL of treatment without 5% S9 activation and levels of 1, 10, 100, 500, or 1000 µg/mL with S9 activation. Relative survival ranged from approximately 20-100%. Statistical analysis of these data revealed no significant increases in mutation frequencies as compared to negative controls at any crude BHMT concentration, and no significant dose response. These results were validated by conducting a triplicate confirmatory assay at the same concentrations and under the same conditions as the initial assay.

Both positive control materials elicited expected responses, confirming the sensitivity of the assay to known mutagens.

Reference: Pharmakon Research International (1985). Report No. PK-314-MO-008-84 "CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay" (June 26), Sponsored by Monsanto.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* CHO/HGPRT Mammalian Cell Gene Mutation Assay: None Found

Type: ***In vivo* Bone Marrow Chromosome Aberration Study in Rats**

Species/Strain: Rats/Sprague-Dawley

Sex/Number: Male and female/15 per sex per dose group

Route of Administration: Gavage

Concentrations: 0, 500 mg/kg

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Crude BHMT was tested to determine its potential for inducing chromosomal damage as measured by numerical and structural chromosome aberrations in bone marrow cells taken from Sprague-Dawley rats. Dosing solutions were prepared with corn oil as the suspension medium.

For the definitive assay, groups of 15 Sprague-Dawley (from Charles River Laboratories) rats/sex received either negative control solutions or 500 mg/kg crude BHMT. Dose levels were selected based on an oral range-finding study. Dosing was by corn oil gavage to prevent corrosion of the stomach. Five rats/sex received the positive control cyclophosphamide at 40 mg/kg intraperitoneally. Body weights were recorded pre-dosing and at 22 and 46 hours, just prior to colchicine administration to surviving animals. Observations of clinical signs of toxicity were recorded twice daily.

All animals on test were administered colchicine at 2.0 mg/kg intraperitoneally 2 hours prior to sacrifice. The positive controls were sacrificed at 24 hours after treatment.
Five rats/sex from each of the other groups were sacrificed at 6, 24, and 48 hours after treatment. Both femurs from each rat were processed for cytogenetic analysis. Two slides were made for each animal. Coded slides were read from each sacrifice interval from 5 rats/sex. Whenever possible, 50 metaphase cells were examined from each rat on study.

The following was recorded for each animal scored: numbers and types of chromosome aberrations, mitotic index, chromosome number per metaphase, and vernier location of each metaphase with chromosome damage. Upon completion of scoring, slides were decoded and data were entered for the appropriate animal and group. Mean mitotic indices, mean chromosome numbers, percent aberrant cells, and mean number of aberrations per cell for each treatment group were compared to the negative controls by Kruskal-Wallis non-parametric statistical tests.

GLP: Yes

Test Substance: Crude BHMT. Material was described by the testing laboratory as a "brown solid" with a purity of 65%. For dosing purposes, the material was assumed to be 65% pure and was adjusted to 100%.

Remark: Based on other test materials sent by this sponsor and the description, it is concluded that this is crude BHMT containing approximately 65% BHMT, 25% Tars and 5-10% other amines on a dry-weigh basis. The water content is unknown but typically is in the 45-50% range.

Results: Negative

Remarks: Because of the corrosive nature of the test material, administration was performed in corn oil to limit excessive mortality from gastric ulcerations.

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Range Finding Study: The test material was evaluated for acute oral toxicity in male and female rats to determine doses for the definitive bone marrow chromosome study. Groups of two animals/sex/group received a single dose of 50, 150, or 500 mg/kg BHMT in isotonic saline, and 250, 500, 1,000, or 2000 mg/kg BHMT in corn oil and observed for one day. Abnormal clinical observations were observed at all levels except 50 mg/kg and loss of body weight was observed at levels of 500 mg/kg and above. The test material produced no apparent effect on the mitotic indices of the animals at 250 or 500 mg/kg. Based on the results of this study, and after consultation with the sponsor, 500 mg/kg was chosen as the highest dose to be tested in the bone marrow study.

One male and 1 female died while on study after being given 500 mg/kg crude BHMT. A variety of clinical signs of toxicity were observed in treated rats. These included depressed motor activity, labored breathing and wheezing, soft feces, and red stains around nose/eyes. Significant body weight depressions were found in treated rats of both sexes at 24 and 48 hours in comparison to controls. The positive control females also experienced a body weight depression.

No significant differences between the mean chromosome numbers or mean mitotic indices of negative controls versus the dosed compound groups were seen. Comparison of the negative control and test material treatment groups revealed no significant differences in the frequency of chromosome aberrations or percent aberrant cells. The cyclophosphamide-treated controls exhibited a significant increase in the average number of aberrations, percent of cells with aberrations, decreased mitotic index, and decreased chromosome number, confirming the sensitivity of the assay to known mutagens.

Time	Group	% Cells Aberrant	# Aberrations per Cell
6 Hours	Control	0.6	0.006
	BHMT 500 mg/kg	0.20	0.002
24 Hours	Control	0.2	0.002
	BHMT 500 mg/kg	0.00	0.000
	Cyclophosphamide	25.0*	1.23*
48 Hours	Control	0.40	0.004
	BHMT 500 mg/kg	0.22	0.45
	* = $p < 0.05$		

Reference:	Hazleton Biotechnologies Corp. (1985). Report No. HL-84-218, "*In vivo* Rat Bone Marrow Chromosome Study BHMT – Bis (Hexamethylene Triamine" (January 31), Sponsored by Monsanto Co.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for *In vivo* Genetic Toxicity: None Found

<u>(February 11, 2003)</u>

APPENDIX B

(February 11, 2003)

Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as related references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

1.0 Substance Information

CAS Number: 143-23-7

Chemical Name: 1,6-Hexanediamine, N-(6-aminohexyl)-

Structural Formula: $H_2N-(CH_2)_6-NH-(CH_2)_6-NH_2$

Other Names:
1,13-Diamino-7-azatridecane
7-Aza-1,13-tridecanediamine
Bis(6-aminohexamethyl)amine
Bis(6-aminohexyl)amine
Bis(hexamethylene)triamine
BHMT
Dihexylamine, 6,6'-diamino-
Dihexylenetriamine

Exposure Limits: None available.

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	33-36°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Aldrich Handbook of Fine Chemicals and Laboratory Equipment (2002-2003). pp. 226, Sigma-Aldrich Fine Chemicals, Saint Louis, MO.
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point:

DuPont Co. (1997). Material Safety Data Sheet No. 6080CR (April 15).

2.2 Boiling Point

Value:	220°C
Decomposition:	No Data
Pressure:	20 mm Hg
Method:	No Data
GLP:	Unknown
Reference:	Aldrich Handbook of Fine Chemicals and Laboratory Equipment (2002-2003). pp. 226, Sigma-Aldrich Fine Chemicals, Saint Louis, MO.
Reliability:	Not assignable because limited study information was available.

Additional References for Boiling Point:

SRC PhysProp data base. http://esc.syrres.com/interkow/PhysProp.htm.

DuPont Co. (1997). Material Safety Data Sheet No. 6080CR (April 15).

2.3 Density: No Data.

2.4 Vapor Pressure

Value:	82.5 mm Hg
Temperature:	250°C
Decomposition:	No Data
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1997). Material Safety Data Sheet No. 6080CR (April 15).
Reliability:	Not assignable because limited study information was available.

Additional References for Vapor Pressure: None Found.

2.5 Partition Coefficient (log Kow)

Value:	1.8 -0.63 (ionized form)
Temperature:	25°C
Method:	Modeled: KOWWIN, v.1.66 module of EPIWIN 3.05 (Syracuse Research Corporation).
GLP:	Not Applicable
Reference:	Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.
Reliability:	Estimated value based on accepted model.

Additional References for Partition Coefficient (log Kow): None Found.

2.6 Water Solubility

Value:	10.8 g/L (Water solubility will vary depending on the water pH and buffering capacity.)
Temperature:	25°C
pH/pKa:	Estimated pKa: terminal amines – 10.2, 2^{o} amine – 10.6
Method:	Modeled: Water solubility: WSKOW, v. 1.4, module of EPIWIN 3.05; pKa: SPARC
GLP:	Not Applicable
Reference:	Solubility: WSKOW v.1.40 in EPIWIN v3.05 (Syracuse Research Corporation). Meylan, W. M. et al. (1996). Environ. Toxicol. Chem., 15:100-106. pKa: SPARC on-line calculator, University of Georgia, http://ibmlc2.chem.uga.edu/sparc/index.cfm.
Reliability:	Estimated value based on accepted model.

Additional References for Water Solubility: None Found.

3.0 Environmental Fate

3.1 Photodegradation:

Concentration:	No Data
Temperature:	No Data
Direct Photolysis:	BHMT is not expected to be subject to aqueous photodegradation.
Indirect Photolysis:	Atmospheric degradation: AOP Program (v1.90) Results:

SMILES : NCCCCCCNCCCCCCN
CHEM : BHMT
MOL FOR: C12 H29 N3
MOL WT : 215.39

SUMMARY (AOP v1.90): HYDROXYL RADICALS
Hydrogen Abstraction = 54.0405×10^{-12} cm^3/molecule-sec
Reaction with N, S and -OH = 105.0000×10^{-12} cm^3/molecule-sec
Addition to Triple Bonds = 0.0000×10^{-12} cm^3/molecule-sec
Addition to Olefinic Bonds = 0.0000×10^{-12} cm^3/molecule-sec
Addition to Aromatic Rings = 0.0000×10^{-12} cm^3/molecule-sec
Addition to Fused Rings = 0.0000×10^{-12} cm^3/molecule-sec

OVERALL OH Rate Constant = 159.0405×10^{-12} cm^3/molecule-sec

	HALF-LIFE = 0.067 Days (12-hr day; $1.5x10^6$ OH/cm^3)
	HALF-LIFE = 0.807 Hours
Breakdown Products:	No Data
Method:	Inspection of chemical structure and AOP program.
GLP:	Not Applicable
Reference:	Harris, J. C. (1990). Rate of Aqueous Photolysis. Chapter 8 In Lyman, W. J. et al. (eds.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC. AOP v1.90 computer program as found in EPIWIN 3.05, Syracuse Research Corporation, Syracuse NY.
Reliability:	Estimate based on known qualitative structure-activity relationships.

3.2 Stability in Water

Concentration:	Not Applicable
Half-life:	Not Applicable
% Hydrolyzed:	The BHMT component of the crude BHMT is not expected to readily hydrolyze in water.
Method:	Inspection of chemical structure.
GLP:	Not Applicable
Reference:	Harris, J. C. (1990). Rate of Aqueous Hydrolysis. Chapter 7 In Lyman, W. J. et al. (eds.). Handbook of Chemical Property Estimation Methods, American Chemical Society, Washington, DC.
Reliability:	Estimated value based on accepted model using established chemical principles.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media:	Air, Water, Soil, and Sediments
Distributions:	NEUTRAL FORM Input Values

Henry's Law	$1.73x10^{-13}$ atm-m^3/mole
Vapor	0.000459 mm Hg
Liquid Vapor	0.00057 mm Hg (super-cooled)
Melting Point	34.5°C (user-entered)
Log Kow	1.8 (KOWWIN program)

Log Kow	1.8 (KOWWIN program)
Soil Koc	25.9 (calc by model)

Medium	% of total	½ life, h	Hypothetical emission to medium, kg/h
Air:	0	2.4	1000
Water:	25.2	360	1000
Soil:	74.7	720	1000
Sediment:	0.1	3240	0

AMMONIUM FORM, with Log Kow adjusted to pH 7
Input Values

Henry's Law Constant	1.73×10^{-13} atm-m^3/mole (HENRYWIN program)
Vapor Pressure	0.000459 mm Hg (MPBPWIN program)
Liquid Vapor Pressure	0.00057 mm Hg (super-cooled)
Melting Point	34.5°C (user-entered)
Log Kow	-1.6 (KOWWIN program)
Soil Koc	0.0103 (calc by model)

Medium	% of total	½ life, h	Hypothetical emission to medium, kg/h
Air	0	1.61	1000
Water	39	360	1000
Soil	60.9	720	1000
Sediment	0.1	3240	0

Adsorption Coefficient:	No Data
Desorption:	No Data
Volatility:	Henry's Law Constant: 1.73×10^{-13} atm-m^3/mole; Group Method
Method:	Modeled: Mackay, Level III, in EPIWIN v3.05 (Syracuse Research Corporation). Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA model defaults.
GLP:	Not Applicable
Reference:	Syracuse Research Corporation EPIWIN v3.05 contains a Level III fugacity model. The methodology and programming approach were developed by Dr. Donald MacKay and coworkers and are detailed in:

	Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press.
	Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.
	Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.
Reliability:	Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation

Value:	The theoretical oxygen demand (ThOD) was calculated as 4.198 mg O_2/mg test substance. For the test substance, the following biodegradability was found: 1% after 7 days, 49% after 14 days, 100% after 17 days, 21 days, and 28 days. Additional nitrate measurements in the 28-day samples confirmed a total nitrification of the test substance during the test period. Therefore, the test substance was classified as ready biodegradable according to the guidelines.
Breakdown Products:	No Data
Method:	The biochemical degradability of the test substance at 293 K (20°C) was determined based on the recommendations of the following guideline: OECD Guideline No. 301 D (which corresponds to the EC-Method, Part C.4, Part E): "Ready Biodegradability: Closed Bottle Test". The mineral nutrient solution was prepared according to the prescriptions of the guideline, the inoculum was a composite made from equal parts of the total effluent and the reflux of an activated sludge. The oxygen depletion was measured after 7, 14, 17, 21, and 28 days by means of an oxygen electrode. Control- and blank-series without test substance were run simultaneously and the effectiveness of the

	inoculum was confirmed (in a 3rd series with sodium acetate as the reference substance) and found to be 102% after 28 days under the conditions of the test.
GLP:	Yes
Test Substance:	BHMT-HP Polyamine, purity >92% by wt.
Reference:	NATEC Institut (1997). Study Number NA 96 9410/3.2 "Biochemical Degradability: BHMT-HP – Polyamine" (June 5).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Biodegradation: None Found.

3.5 Bioconcentration

Value:	Estimated BCF = 4.8 (Neutral Form) Estimated BCF = 3.2 (Ionized Form, water pH 7, model default for log Kow <1).
Method:	Modeled: BCFWIN v. 2.14 module of EPIWIN v3.05 (Syracuse Research Corporation). The log Kow value used to make the BCF estimate was 1.8.
GLP:	Not Applicable
Reference:	The estimation methodology used by BCFWIN is described in the following document prepared for the U.S. Environmental Protection Agency (OPPT): "Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient," SRC TR-97-006 (2nd Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-00012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil Gouchie; Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.
Reliability:	Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**48-hour LC_{50}**
Species:	Orfe, *Leuciscus idus melanotus*
Value:	76 mg/L
Method:	The procedure used in the test was based on the

recommendations of the following guideline:

German Standard "Deutsches Einheitsverfahren" DIN 38 412, Part 15.

The effects of the test substance on *Leuciscus idus melanotus* within 48 hours were examined and compared to a negative control. Ten fish were exposed to various concentrations of the test substance (0, 18, 32, 58, and 100 mg/L) in water without using any solubilising agent. The test was performed using a static test procedure. Two hours before test start, 15 L of each concentration was prepared and distributed in the test containers. These solutions were stored under test conditions until the test started. After this precoating time, the solutions were replaced by freshly prepared solutions and 10 animals were introduced into the test containers (1 test container for each concentration).

The main test criteria were the mortalities after 24 and 48 hours in each solution. Those animals showing no reaction within a few seconds after touching the caudal peduncle were considered to be dead. Dead animals were removed at the observation times and test containers were exchanged and cleaned. Other significant effects compared to the control observed in the test containers were also documented.

Ambient air was pumped by means of an aquarium aerator through silicon rubber tubes and "flow-out stones" into the aquaria. The air flow was adjusted in order to maintain an oxygen concentration of >80% of the saturation. No feeding occurred during the test. Sixteen hours of light and 8 hours of darkness were provided during the test. Water hardness was 2.2 mole $CaCO_3$/L.

The dilution water was aerated until a constant pH value (8.0±0.5) was reached.

GLP: Yes

Test Substance: BHMT, purity >92% by wt.

Results: After evaluation of the results with a "Probit" method, the following toxicity data were obtained:

NOEC = 58 mg/L (nominal);
0% mortality at 58 mg/L (nominal);
LC_{50} = 76 mg/L (nominal);
100% mortality = 100 mg/L (nominal).

	No other effects of the test substance compared to the control were observed.
	The pH was 8.4 (concentration not specified) when measured at 0.1, 24, and 48 hours. The oxygen content (% of saturation) was 92% at 0.1, 24, and 48 hours. The water temperature was 20.1, 20.2, and 20.2°C at 0.1, 24, and 48 hours, respectively.
Reference:	NATEC Institut (1997). Study Number NA 96 9410/3.3 "Acute Toxicity Test on the Orfe (*Leuciscus idus melanotus*) – Static Test Procedure, 48-Hours: BHMT-HP – Polyamine" (June 3).
Reliability:	Medium because a suboptimal study design was used. Only nominal concentrations were reported.

Type:	**96-hour LC_{50}**
Species:	Freshwater fish
Value:	79.6 mg/L
Method:	Modeled, ECOSAR (using log Kow of 1.80)
GLP:	Not Applicable
Test Substance:	BHMT
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). <u>User's Guide for the ECOSAR Class Program</u>, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates

Type:	**48-hour EC_{50}**
Species:	Daphnid
Value:	5.7 mg/L
Method:	Modeled, ECOSAR (using log Kow of 1.80)
GLP:	Not Applicable
Test Substance:	BHMT
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). <u>User's Guide for the ECOSAR Class Program</u>, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S.

	Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants

Type:	**96-hour EC_{50}**
Species:	Green algae
Value:	10.1 mg/L
Method:	Modeled, ECOSAR (using log Kow of 1.80)
GLP:	Not Applicable
Test Substance:	BHMT
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral ALD**
Species/Strain:	Male rats/Crl:CD®BR
Value:	1500 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. The test substance was suspended in corn oil and administered to 1 rat per dose level by intragastric intubation. Dose levels used in the study included 200, 450, 670, 1000, 1500, 2300, and 3400 mg/kg. Male rats were 7 weeks of age when received for the study and approximately 8 weeks at test substance administration.

	Following administration of the test substance, rats were observed for clinical signs of toxicity. Surviving rats were weighed and observed daily until signs of toxicity subsided, and then at least 3 times a week throughout the 14-day observation period. Observations for mortality and signs of illness, injury, or abnormal behavior were made daily throughout the study. Pathological examinations were not performed.
GLP:	No
Test Substance:	BHMT, purity >95%
Results:	The rats administered 1500, 2300, and 3400 mg/kg were found dead the day after dosing. Other than death, no clinical signs of toxicity were observed in the rat dosed with 1500 mg/kg. The rat dosed with 2300 mg/kg exhibited lethargic behavior, low carriage, low posture, labored breathing, wet perineum, and weight loss of approximately 5% of initial body weight by the day after dosing. The rat dosed at 3400 mg/kg exhibited lethargic behavior, low carriage, and low posture the day of dosing. At non-lethal doses, no clinical signs of toxicity were observed at 200 mg/kg. The rat dosed at 450 mg/kg had weight loss of approximately 2% of initial body weight, and the rat dosed at 670 mg/kg had weight loss of approximately 8% of initial body weight by the day after dosing. The rat dosed at 1000 mg/kg exhibited lung noise, ruffled fur, yellow-stained perineum, and weight loss of approximately 18% of initial body weight by 2 days after dosing.
Reference:	DuPont Co. (1996). Unpublished Data, Haskell Laboratory Report No. 814-95, "Approximate Lethal Dose (ALD) in Rats" (February 8).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Oral Toxicity: None Found.

Type: **Inhalation Toxicity:** No Data.

Type: **Acute Dermal Toxicity:** No Data.

Type: **Dermal Irritation:** No Data.

Type: **Dermal Sensitization:** No Data.

Additional Reference for Dermal Sensitization:

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

Anon. (1989). TSCA Fiche OTS0000678.

Type: **Eye Irritation:** No Data.

5.2 **Repeated Dose Toxicity:** No Data.

5.3 **Developmental Toxicity:** No Data.

5.4 **Reproductive Toxicity:** No Data.

5.5 **Genetic Toxicity**

Type:	***In vitro* Bacterial Reverse Mutation Assay**
Tester Strain:	*Salmonella typhimurium* strains TA97a, TA98, TA100, and TA1535 *Escherichia coli* strain WP2 *uvr*A (pKM101)
Exogenous Metabolic Activation:	With and without Aroclor-induced rat liver S-9 homogenate
Exposure Concentrations:	0, 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate
Method:	The procedures used in the test were based on the recommendations of the following guidelines: U.S. EPA OPPTS Guidelines, Subpart H, 40 CFR Part 870.5100 [1998] OECD Guideline 471 [1997] EEC Commission Directive 92/69/EEC, Methods B.13 and B.14 [1992] Ministry of Labour (MOL) Guideline for Screening Toxicity of Chemicals, Section III. 1. Reverse Mutation Assay in Bacteria, Japan [1986]. Sterile water was chosen as the test substance solvent, diluent, and negative control. Positive control substances used in the test included 2-nitrofluorene (2NF), N-ethyl-N-nitro-N-nitroguanidine (ENNG), sodium azide (NAAZ), ICR 191 acridine mutagen (ICR 191), 9,10-dimethyl-1,2-benzanthracene (DMBA), and 2-aminoanthracene (2AA).

(February 11, 2003)

This study consisted of a single trial that assessed test substance mutagenicity. Three replicates were plated for each tester strain in the presence or absence of the exogenous metabolic activation system at each test substance concentration. Positive and negative controls were included for each strain and condition. Treatments with the exogenous metabolic activation were conducted by adding negative or positive control or test substance, S9, and an overnight culture containing 1×10^8 bacteria to top agar. The S9 mixture contained S9, glucose 6-phosphate, $NADP^+$, $MgCl_2$, and KCl in a sodium phosphate buffer. The components were briefly mixed, and poured onto the surface of a minimal glucose agar plate. Treatments in the absence of metabolic activation were the same as those in the presence of activation with the exception that sterile phosphate buffer was used as a replacement for the volume of the exogenous metabolic activation system. After the pouring onto the surface of the minimal glucose agar plates, the top agar was allowed time to solidify, and the individually labeled plates were inverted and incubated for approximately 48 hours at 37°C. When necessary, plates were refrigerated at approximately 4°C prior to evaluation and counting of revertant colonies.

Bacterial background lawn were evaluated for evidence of test substance toxicity and precipitation. Evidence of toxicity was scored relative to the concurrent negative control plates recorded with the mean revertant count for the strain, condition, and concentration. Revertant colonies for a given tester strain and condition were counted by an automated colony counter.

Data for each tester strain were evaluated independently. For each tester strain, the mean and standard deviation of the number of revertants per plate were calculated. A test substance was classified positive if the mean number of revertants in any strain at any test concentration was at least 2 times greater than the mean of the concurrent vehicle control and there was a concentration-related increase in the mean revertants per plate in that same strain. A test substance was classified as negative if there were no test substance concentrations with a mean number of revertants that was at least 2 times greater than the mean of the concurrent vehicle control, or there was no concentration-related increase in the mean revertants per plate in that same strain.

GLP:	Yes
Test Substance:	BHMT, purity 97.1%
Results:	Negative
Remarks:	No test substance-related precipitate was observed in any concentration in any of the *Salmonella* strains or in the *E. coli* strain. Test substance-related toxicity was observed in the background lawns as a reduction in the number of revertant colonies of all plated strains as low as 500 µg/plate without metabolic activation, and as low as 1000 µg/plate with metabolic activation. There were no test substance concentrations with a mean number of revertants that was 2 times greater than the mean of the concurrent vehicle control, nor was there a concentration-related increase in the mean revertants per plate in any strain.
Reference:	DuPont Co. (1999). Unpublished Data, Haskell Laboratory Report No. DuPont-2202, "Bacterial Reverse Mutation Test" (April 5).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for *In vitro* Bacterial Reverse Mutation Assay: None Found.

Type: ***In vitro* Clastogenicity Studies:** No Data.

Type: ***In vivo* Genetic Toxicity:** No Data.